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COPOLYMERS FOR USE IN CAPILLARY ELECTROPHORETIC SEPARATION MEDIA

Background of the Invention

[0001] The present invention relates generally to polymer formulations for use in separation media, where the polymer formulation comprises either a block copolymer or a graft polymer wherein one segment or block is compatible with a wall-coating function and a second segment or block is compatible with a sieving function. In some embodiments, the copolymer comprises the polymer formulation, in other embodiments the copolymer functions primarily as the wall-coating agent and in still other embodiments, the copolymer is used in addition to a wall coating polymer and a sieving polymer. In preferred embodiments, the invention comprises a block copolymer of N,N-dimethylacrylamide and acrylamide. This invention further is related to separation media comprising such copolymers and to separation applications for such media.

[0002] Electrophoresis is a technique used for separation and analysis of charged molecules such as biopolymers (e.g., nucleic acid polymers such as DNA, RNA and amino acid polymers such as proteins). Typically, one or more samples containing molecules to be separated or analyzed are loaded onto a separation media, and a voltage is applied across the media. The applied voltage causes the charged molecules to move differentially, thereby fractionating the sample into its various components. See for example U.S. Patents 5,126,021, 5,631,337 and 5,883,211. Karger et al. have demonstrated the utility of linear polyacrylamide polymers for DNA sequencing, with the polyacrylamide typically being part of a formulation that includes polymers of different molecular mass. See Karger et al., *Anal. Chem.*, 1998, 70, 3996-4003; *Anal. Chem.*, 1998, 70, 1516-1527; *Electrophoresis*, 1998, 19, 242-248; *Anal. Chem.*, 2000, 72, 1045-1052; and *Anal. Chem.*, 1996, 68, 3305-3313.

[0003] Gel-slab electrophoresis is well known, but more typical capillary electrophoretic separation approaches have been automated and are widely employed. However, capillary electrophoretic approaches have limitations with respect to sample component resolution. Known separation media for capillary gel electrophoresis typically comprise linear molecular chains that may become entangled and thereby impart a mesh-like characteristic to the separation media. Block copolymers have been

suggested for this separation media, however, in these suggestions have been made in order to modify the mesh characteristics. For example, U.S. Patents 5,290,418 and 5,468,365 to Menchen et al. discuss various polymeric separation media, including block polymers of hydrophobic and hydrophilic segments, with the intention that the block copolymers form meshes. More recently, Song *et al.* have suggested networks of copolymers of polyacrylamide and polyvinylpyrrolidone, *Electrophoresis*, 2001, 22, 3688-3698.

[0004] The coating of the interior of the silica capillary in a capillary electrophoretic separation system is known to reduce electro-osmotic flow (EOF) in electrophoresis. EOF is produced as a consequence of the electric charges that build up at the silica surface. These charges result from the deprotonation of the surface silanol groups when the pH is in the neutral-basic region typical of capillary electrophoresis separation of nucleic acids. These superficial charges produce a counter-ion cloud close to the capillary wall that travels to the cathode in a flow inverse to the electrophoretic flow of the DNA. This counter-flow is often heterogeneous along the capillary as it reflects the surface charge density fluctuation at the silica surface, and thus contributes to broaden the residence time distribution of the DNA. This can translate into broader peaks and lower resolution.

[0005] Others have applied either a permanent coating (i.e., a static coating) to the capillary inner walls or a dynamic coating that temporarily coats the inner capillary walls. A dynamic coating, generally, comprises a component of the separation media that interacts with the capillary wall, effectively "coating" the wall. Also, since static coated capillaries are generally expensive, most commercial systems employ un-coated capillaries, and the separation media formulation includes a dynamic wall-coating component. Others have also provided a dynamic coating that comprises dimethylacrylamide polymer in separation media formulations, see, e.g., U.S. Patent 5,567,292, which explains that coating the wall of un-coated capillaries reduces analyte-wall interactions. See also, U.S. Patents 5,552,028, 6,074,542 and 5,948,227.

[0006] Unfortunately, simply combining wall coating polymers and sieving polymers has led to incompatibilities between the components of the separation media. These incompatibilities lead to at least micro-phase separation (and possibly more severe phase separation) between the components. This degradation in the separation media has

resulted in a less effective separation media. The phase separation reduces the shelf life of the separation media and the resultant difficulties in marketing such separation media. Moreover, remixing media that has phase separated does not necessarily result in a homogeneous mixture because the separated phases may co-acervate. Upon remixing, a heterogeneous mixture forms that provides inconsistent results in a capillary electrophoresis system.

[0007] Others have recognized incompatibility, but have recommended solutions that are considered impractical, including use of low molecular weight PDMA and a low concentration of the PDMA wall-coating polymer. Song *et al.*, *J. Chromatography A*, 915 (2001), 231-239. In particular, it has been found that the lower molecular weight PDMA of about 8000 is not as good as a wall-coating polymer as higher molecular weight PDMA. See, commonly owned, co-pending U.S. Provisional Application No. 60/298,463, filed June 13, 2001, which is incorporated herein by reference.

[0008] This invention solves the compatibility problems by providing a copolymer having blocks or segments that are compatible with the different phases and/or functions of the separation media, including both the wall-coating and sieving. In preferred embodiments, the wall-coating polymer itself is a block copolymer or a graft copolymer comprising a block (*e.g.*, segment or graft) that has the same chemical composition as the sieving polymer.

Summary of the Invention

[0009] It is, therefore, an object of the invention to provide separation media having improved separation capabilities. It is also an object of the invention to provide a separation media having components for both wall-coating and sieving, where the components are compatible with each other.

[0010] The present invention alleviates the current limitations encountered in blend formulation of polymers as separation media in capillary electrophoresis of DNA. The new composition comprises at least a polymer that has sieving properties, which is connected to distinct polymer segments that have wall coating properties, said composition does not exhibit substantial phase separation and keep its separation performance for a prolonged period of time (*e.g.*, has a long shelf life). The compositions of the presenting invention comprise a polymer that is either a block or

graft copolymers that contains segment A and segment B, with A and B having sieving and wall-coating properties, respectively. The polymers of this invention may be generally characterized by one or more of the following formulas:

$((A)-(B))_n-A_m$ Formula 1;

5 $(A)-(B)_n$ Formula 2;

$(B)-(A)_n$ Formula 3.

Formula 1 represents a block copolymer with blocks of A and B, wherein n is the number of A blocks connected to B blocks and m is the number of A blocks attached at one end or the other of the blocks. n is typically in the range of 1-5 and m is typically 1 or 2. Formula 2 represents a graft copolymer with a polymer backbone A having n' polymer segment B radiating from A. Formula 3 represents a graft copolymer with a polymer backbone B with n' polymer segment A radiating from B. n' is typically in the range of 1 to 50.

[0011] In one embodiment of this invention, the separation media comprises a polymer represented by either of Formulas 1, 2 or 3 (or a combination thereof) as the polymeric component in the separation media. In another embodiment the separation media comprises a polymer formulation comprising sieving polymer and a polymer represented by either of Formulas 1, 2, 3 or a combination of these polymers. In this embodiment, the sieving polymer is preferably prepared from the same monomers as those that comprise the A block or segment. In still another embodiment the separation media comprises a polymeric formulation comprising a wall-coating polymer and a polymer represented by either of Formulas 1, 2, 3 or a combination of these polymers. In this embodiment, the wall-coating polymer is preferably prepared from the same monomers as those that comprise the B block or segment. In yet another embodiment the separation media comprises a polymer formulation comprises a sieving polymer, a wall coating polymer and a polymer represented by either of Formulas 1, 2, 3 or a combination of these polymers. Similarly, in this embodiment, the sieving polymer is preferably prepared from the same monomers as those that comprise the A block or segment and the wall-coating polymer is preferably prepared from the same monomer(s) as those that comprise the B block or segment.

[0012] Other features, objects and advantages of the present invention will be apparent to those skilled in art and in part pointed out hereinafter. All references cited in

the instant specification are incorporated by reference for all purposes. Moreover, as the patent and non-patent literature relating to the subject matter disclosed and/or claimed herein is substantial, many relevant references are available to a skilled artisan that will provide further instruction with respect to such subject matter.

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Detailed Description

[0013] According to the present invention, controlled-architecture polymers have properties that are advantageous for separation systems, and especially for
10 electrophoretic separation systems such as capillary gel electrophoresis. Separation media comprising such polymers, individually or as blends, can be tailored for a particular separation objective of interest. Moreover, separation media of low viscosity, but suitable resolution, can be advantageously applied to increase the read-length for biological polymer samples (*e.g.*, DNA), to improve the sample throughput, to reduce the
15 dimensions of the capillary gel electrophoresis systems and/or to improve heat-transfer.

[0014] Without wishing to be bound by any particular theory or mechanism, when a neutral thin polymer layer is deposited at the capillary surface the EOF is greatly reduced as a result of the ionizable sites being hidden from the running buffer or because of the large increase of viscosity at the vicinity of the surface that slow down or simply
20 inhibit the counter ions counter flow. The polymer coating is either provided through covalent attachment or by dynamic adsorption. In the latter case the wall-coating polymer is either used in a pre-coating step whereby the wall coating polymer is flushed in the pristine capillary, then equilibrated and washed away, or it can be solubilized in the separation medium. The dynamic wall-coating presents a cost and practical
25 advantage over the covalent coupling approach, as it does not require a lengthy chemical coupling procedure and gives higher run to run consistency. This better stability is thought to be due to the dynamic exchange of the wall-coating polymer between the bulk of the separation medium and the surface that insures a more consistent capillary surface coverage upon use.

30 [0015] In some cases the wall coating polymer and the sieving polymer are the same entity. For example, polydimethylacrylamide (PDMA) provides both dynamic wall coating and sieving properties. In contrast, linear polyacrylamide (LPA), while a good sieving polymer, is not known to dynamically coat the capillary surface nor lead to

good separation in uncoated capillary when used alone. The use of blends of polymers where one component acts as the sieving polymer and the other minimizes the EOF by its wall coating properties has already been described.

5 [0016] However, while investigating a straightforward blending approach it was found that simple blends, despite being carefully selected, failed to be stable over the long time storage required for commercialization (and despite giving high separation resolutions). It was found that after several weeks or months (depending upon the formulation), the separation media showed a net degradation of its performance and in some instances the separation media showed signs of phase separation after being aged
10 several weeks. In the worst case the solution of the polymer mixture turned turbid after only one day, which signaled a relatively prompt phase separation. Upon further investigation, it was found that phase separation was typically accompanied by a loss of separation performance. Moreover, phase separation was often irreversible; meaning that when the separation media polymer blend was re-homogenized (*e.g.*, by gently
15 rolling the polymer solution), the separation performance remained markedly lower as compared to freshly prepared separation media.

 [0017] Here, it is disclosed that the use of polymers combining wall-coating and sieving functions (*e.g.*, blocks or segments) in a controlled architecture solved the reduced separation performance.

20 [0018] In other embodiments of this invention, it has been found that separation media comprising the polymer formulations of the present invention (*e.g.*, a blend of 2% LPA as a sieving polymer and a block copolymer of polydimethylacrylamide-*b*-polyacrylamide), provide consistent separation performance with increasing amounts of the wall coating polymer (*e.g.*, diblock) in the polymer formulation in the separation
25 media from 0.1% to 0.6%, while a severe loss of performance is visible in the blend polyacrylamide / polydimethylacrylamide when the wall coating material (polydimethylacrylamide) is increased in the same proportion. In the latter case the degradation most likely reflects the onset of phase separation. This also illustrates the much higher degree of flexibility offered by the polymers of the invention in formulating
30 separation media over the polymer systems known from the prior art.

 [0019] In still other embodiments of this invention, it has been found that the polymer formulations of the present invention can provide both functions of wall-coating

and sieving, thereby eliminating the need to blend polymers in a separation media. In these embodiments the polymer segment that provides the sieving function is polymerized to a relatively high molecular weight (up to about 2,000,000), while the polymer segment(s) or block(s) that provide the wall-coating function can be kept to a relatively low molecular weight (such as each segment or block having a molecular weight below about 500,000, more specifically below about 200,00 and even more specifically below about 150,000). The polymers in this embodiment can be either block polymers or grafts, as described in the formulas herein.

[0020] As used herein, the phrase "characterized by the formula" is not intended to be limiting and is used in the same way that "comprising" is commonly used. The term "independently selected" is used herein to indicate that the R groups, e.g., R^1 , R^2 and R^3 can be identical or different (e.g. R^1 , R^2 and R^3 may all be substituted alkyls or R^1 and R^2 may be a substituted alkyl and R^3 may be an aryl, etc.). A named R group will generally have the structure that is recognized in the art as corresponding to R groups having that name. For the purposes of illustration, representative R groups as enumerated above are defined herein. These definitions are intended to supplement and illustrate, not preclude, the definitions known to those of skill in the art.

[0021] The term "alkyl" is used herein to refer to a branched or unbranched, saturated or unsaturated acyclic hydrocarbon radical. Suitable alkyl radicals include, for example, methyl, ethyl, n-propyl, isopropyl, propenyl (or allyl), hexyl, vinyl, n-butyl, *tert*-butyl, iso-butyl (or 2-methylpropyl), etc. In particular embodiments, alkyls have between 1 and 200 carbon atoms, between 1 and 50 carbon atoms or between 1 and 20 carbon atoms.

[0022] "Substituted alkyl" refers to an alkyl as just described in which one or more hydrogen atom to any carbon of the alkyl is replaced by another group such as a halogen, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, and combinations thereof. Suitable substituted alkyls include, for example, benzyl, trifluoromethyl and the like.

[0023] The term "heteroalkyl" refers to an alkyl as described above in which one or more hydrogen atoms to any carbon of the alkyl is replaced by a heteroatom selected from the group consisting of N, O, P, B, S, Si, Se and Ge. The bond between the carbon atom and the heteroatom may be saturated or unsaturated. Thus, an alkyl substituted

with a heterocycloalkyl, substituted heterocycloalkyl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, boryl, phosphino, amino, silyl, thio, or seleno is within the scope of the term heteroalkyl. Suitable heteroalkyls include cyano, benzoyl, 2-pyridyl, 2-furyl, $\text{Me}_3\text{SiOCH}_2(\text{CH}_3)_2\text{C}-$ and the like.

5 **[0024]** The term "cycloalkyl" is used herein to refer to a saturated or unsaturated cyclic non-aromatic hydrocarbon radical having a single ring or multiple condensed rings. Suitable cycloalkyl radicals include, for example, cyclopentyl, cyclohexyl, cyclooctenyl, bicyclooctyl, etc. In particular embodiments, cycloalkyls have between 3 and 200 carbon atoms, between 3 and 50 carbon atoms or between 3 and 20 carbon
10 atoms.

[0025] "Substituted cycloalkyl" refers to cycloalkyl as just described including in which one or more hydrogen atom to any carbon of the cycloalkyl is replaced by another group such as a halogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, heteroaryl,
15 substituted heteroaryl, alkoxy, aryloxy, boryl, phosphino, amino, silyl, thio, seleno and combinations thereof. Suitable substituted cycloalkyl radicals include, for example, 4-dimethylaminocyclohexyl, 4,5-dibromocyclohept-4-enyl, and the like.

[0026] The term "heterocycloalkyl" is used herein to refer to a cycloalkyl radical as described, but in which one or more or all carbon atoms of the saturated or unsaturated
20 cyclic radical are replaced by a heteroatom such as nitrogen, phosphorus, oxygen, sulfur, silicon, germanium, selenium, or boron. Suitable heterocycloalkyls include, for example, piperazinyl, morpholinyl, tetrahydropyranyl, tetrahydrofuranyl, piperidinyl, pyrrolidinyl, oxazolinyl, and the like.

[0027] "Substituted heterocycloalkyl" refers to heterocycloalkyl as just described
25 including in which one or more hydrogen atom to any atom of the heterocycloalkyl is replaced by another group such as a halogen, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, boryl, phosphino, amino, silyl, thio, seleno and combinations thereof. Suitable substituted heterocycloalkyl radicals include, for example, N-methylpiperazinyl, 3-dimethylaminomorpholine, and the like.

30 **[0028]** The term "aryl" is used herein to refer to an aromatic substituent which may be a single aromatic ring or multiple aromatic rings which are fused together, linked covalently, or linked to a common group such as a methylene or ethylene moiety. The

common linking group may also be a carbonyl as in benzophenone or oxygen as in diphenylether or nitrogen in diphenylamine. The aromatic ring(s) may include phenyl, naphthyl, biphenyl, diphenylether, diphenylamine and benzophenone among others. In particular embodiments, aryls have between 1 and 200 carbon atoms, between 1 and 50 carbon atoms or between 1 and 20 carbon atoms.

[0029] "Substituted aryl" refers to aryl as just described in which one or more hydrogen atom to any carbon is replaced by one or more functional groups such as alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, halogen, alkylhalos (e.g., CF₃), hydroxy, amino, phosphino, alkoxy, amino, thio and both saturated and unsaturated cyclic hydrocarbons which are fused to the aromatic ring(s), linked covalently or linked to a common group such as a methylene or ethylene moiety. The linking group may also be a carbonyl such as in cyclohexyl phenyl ketone. Specific examples of substituted aryls include perfluorophenyl, chlorophenyl, 3,5-dimethylphenyl, 2,6-diisopropylphenyl and the like.

[0030] The term "heteroaryl" as used herein refers to aromatic rings in which one or more carbon atoms of the aromatic ring(s) are replaced by a heteroatom(s) such as nitrogen, oxygen, boron, selenium, phosphorus, silicon or sulfur. Heteroaryl refers to structures that may be a single aromatic ring, multiple aromatic ring(s), or one or more aromatic rings coupled to one or more nonaromatic ring(s). In structures having multiple rings, the rings can be fused together, linked covalently, or linked to a common group such as a methylene or ethylene moiety. The common linking group may also be a carbonyl as in phenyl pyridyl ketone. As used herein, rings such as thiophene, pyridine, isoxazole, phthalimide, pyrazole, indole, furan, etc. or benzo-fused analogues of these rings are defined by the term "heteroaryl."

[0031] "Substituted heteroaryl" refers to heteroaryl as just described including in which one or more hydrogen atoms to any atom of the heteroaryl moiety is replaced by another group such as a halogen, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, boryl, phosphino, amino, silyl, thio, seleno and combinations thereof. Suitable substituted heteroaryl radicals include, for example, 4-N,N-dimethylaminopyridine.

[0032] The term "alkoxy" is used herein to refer to the —OZ¹ radical, where Z¹ is selected from the group consisting of alkyl, substituted alkyl, cycloalkyl, substituted

cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, silyl groups and combinations thereof as described herein. Suitable alkoxy radicals include, for example, methoxy, ethoxy, benzyloxy, *t*-butoxy, etc. A related term is "aryloxy" where Z^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl, substituted heteroaryl, and combinations thereof. Examples of suitable aryloxy radicals include phenoxy, substituted phenoxy, 2-pyridinoxy, 8-quinolinoxy and the like.

[0033] As used herein the term "silyl" refers to the $-\text{SiZ}^1\text{Z}^2\text{Z}^3$ radical, where each of Z^1 , Z^2 , and Z^3 is independently selected from the group consisting of alkyl, substituted alkyl, cycloalkyl, heterocycloalkyl, heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, amino, silyl and combinations thereof.

[0034] As used herein the term "boryl" refers to the $-\text{BZ}^1\text{Z}^2$ group, where each of Z^1 and Z^2 is independently selected from the group consisting of alkyl, substituted alkyl, cycloalkyl, heterocycloalkyl, heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, amino, silyl and combinations thereof.

[0035] As used herein, the term "phosphino" refers to the group $-\text{PZ}^n$, where each of Z^n is independently selected from the group consisting of hydrogen oxygen, substituted or unsubstituted alkyl, cycloalkyl, heterocycloalkyl, heterocyclic, aryl, heteroaryl, silyl, alkoxy, aryloxy, amino and combinations thereof, where n is 1 to 4 depending on the phosphorus oxidation state.

[0036] The term "amino" is used herein to refer to the group $-\text{NZ}^1\text{Z}^2$, where each of Z^1 and Z^2 is independently selected from the group consisting of hydrogen; alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, silyl and combinations thereof.

[0037] The term "thio" is used herein to refer to the group $-\text{SZ}^1$, where Z^1 is selected from the group consisting of hydrogen; alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, silyl and combinations thereof.

[0038] The term "seleno" is used herein to refer to the group $-\text{SeZ}^1$, where Z^1 is selected from the group consisting of hydrogen; alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted

aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, silyl and combinations thereof.

[0039] The term “saturated” refers to lack of double and triple bonds between atoms of a radical group such as ethyl, cyclohexyl, pyrrolidinyl, and the like.

[0040] The term “unsaturated” refers to the presence one or more double and
5 triple bonds between atoms of a radical group such as vinyl, acetylenyl, oxazolinyl, cyclohexenyl, acetyl and the like.

[0041] The controlled-architecture polymers of the present invention generally comprise a polymer that is either a block or graft polymer that comprises at least segment A and segment B, with A and B having sieving and wall-coating properties, respectively. In some embodiments, one or more of the following formulas may generally characterize the polymers of this invention:

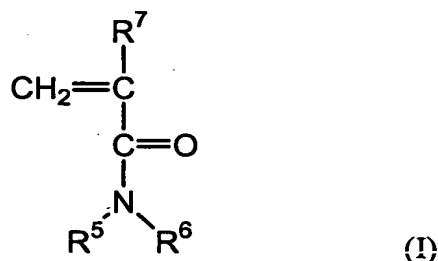
$((A)-(B))_n-A_m$ Formula 1;

$(A)-(B)_{n'}$ Formula 2; or

$(B)-(A)_{n'}$ Formula 3.

Formula 1 represents a block copolymer with blocks of A and B. Formula 2 represents a graft copolymer with a polymer backbone A having n' polymer segment B radiating from A. Formula 3 represents a graft copolymer with a polymer backbone B with n' polymer segment A radiating from B. In Formula 1, n is at least 1, more specifically between 1 and 5, and even more specifically 1, 2 or 3; and m is 0 or 1. In Formulas 2 and 3, n' is at least 1, more specifically a number between 1 and 50 and even more specifically between 3 and 15.

[0042] The monomers that form repeating units to make up the sieving A segment(s) are typically acrylimidic repeat units derived from monomers having the formula I:



where R^7 is H or an alkyl group; and R^5 and R^6 , independently, are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heteroalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, and combinations thereof; optionally, R^5 and R^6 may be joined together in a cyclic ring structure, including heterocyclic ring structure, and that may have fused with it another saturated or aromatic ring. In preferred embodiments, the A segment(s) comprises, or alternatively consist essentially of, the acrylamide-based repeat units derived from monomers such as acrylamide, methacrylamide, N-alkylacrylamide (e.g., N-

methacrylamide, N-*tert*-butylacrylamide, and N-*n*-butylacrylamide), N-alkylmethacrylamide (e.g., N-*tert*-butylmethacrylamide and N-*n*-butylmethacrylamide), N,N-dialkylacrylamide (e.g., N,N-dimethylacrylamide), N,N-dialkylmethacrylamide, N-methylolmethacrylamide, N-ethylolmethacrylamide, N-methylolacrylamide, N-ethylolacrylamide, and combinations thereof. In another preferred embodiment, the A segment comprises acrylamidic repeat units derived from monomers selected from N-alkylacrylamide, N-alkylmethacrylamide, N,N-dialkylacrylamide and N,N-dialkylmethacrylamide. Preferred repeat units can be derived, specifically, from acrylamide, methacrylamide, N,N-dimethylacrylamide, and *tert*-butylacrylamide. Although polymers comprising the acrylamidic repeat units as described above are generally preferred, some embodiments of the present invention have more general application with respect to the repeat unit, and can comprise, or alternatively consist essentially of, repeat units other than the aforementioned acrylamidic repeat units. For example, other sieving A segment(s) may include hydroxyethylcellulose, hydropropylcellulose, and polyethylene oxide.

[0043] The monomers that form repeating units to make up the wall coating B segment(s) comprise, or alternatively consist essentially of, monomers that interact with the Si-OH group of the inner wall of an uncoated capillary in a capillary electrophoresis system. Such monomers may be selected from the group consisting of N,N-dialkylacrylamide (e.g., N,N-dimethylacrylamide), ethyleneoxide and propyleneoxide (and combinations thereof), N-vinyl pyrrolidone, vinyl alcohol, allyl glycidyl ether, and combinations thereof. Combinations of these monomers are typically random copolymers that form the wall coating B segment(s).

[0044] The monomers used to form the repeating units of the sieving A segment and the wall-coating B segment are different.

[0045] The molecular weight of the polymers is not narrowly critical in the general case. The molecular weight is an important property of interest, however, for specific properties. For example, it has been found that in a preferred embodiment the molecular weight of the sieving A segment is between about 5 to 50 times the molecular weight of the wall coating B segment. In some embodiments, more specifically, the molecular weight of the sieving A segment is between about 7 to 15 times the molecular weight of the wall coating B segment.

[0046] The molecular weight of each sieving A block is generally in the range of from about 100,000 to about 3,000,000, more specifically between about 150,000 and about 1,500,000 and even more specifically between about 150,000 and about 750,000. The molecular weight of each wall coating B block is generally in the range of from
5 about 10,000 to about 200,000, more specifically between about 30,000 and about 150,000 and even more specifically between about 30,000 and about 100,000. These and other more specific ranges may be preferred in particular combinations with other polymer properties, as discussed below.

[0047] Molecular weight values can be obtained by size-exclusion
10 chromatography (SEC), based on correlation to narrow linear polystyrene standards. For example, a SEC-observed Mw value of 100,000 means that the measured polymer has the same hydrodynamic volume as the polystyrene of the molecular weight 100,000 under the conditions used for both calibration and characterization (DMF + 0.1% TFA) of all samples. Absolute molecular weights of the first block or of the final product can
15 be obtained with rapid light scattering, which utilizes a combination of fast separation of macromolecules of interest from small molecules (*e.g.*, interferences) with multi-angle light scattering detection (MALS).

[0048] The viscosity of the polymer solution is not critical in the general case, but can be important in some applications – particularly in applications in which flow of
20 the polymer is desired or required. Hence, the viscosity of the polymer should, in general, be suitable for use in the particular application at hand (*e.g.*, capillary gel electrophoresis – discussed in greater detail in connection with separation media therefore).

[0049] The polymers may be soluble or dispersible in water or the running buffer
25 of a capillary electrophoresis system. More specifically, the polymers are at least partially soluble, and are preferably substantially soluble or completely soluble in water or the running buffer. In addition, one block or segment may be water or running buffer soluble, while the other block or segment may be dispersible in water or running buffer. In some embodiments, the polymers of this invention do not include those that have a
30 cloud point (or LCST) under conditions of a capillary electrophoresis separation run, such as those polymers that exhibit a solubility transition in the temperature range typical for a capillary electrophoresis experiment. As used herein, the term “at least partially

soluble” means that at least some amount of the compound of interest is present as a solute in a continuous phase solution medium. The dispersible polymers are preferably uniformly dispersed mixtures of solid polymer particles in a liquid, preferably aqueous, continuous phase. The size of the particles in such a dispersion is, in preferred applications, small enough to form a stable dispersion. Water-soluble and water-dispersible polymers have numerous applications and can provide numerous advantages in such applications. For polymers employed in capillary gel electrophoresis, the polymers are preferably water soluble or water dispersible as described, but can, less stringently, be soluble (at least partially soluble) or dispersible (preferably uniformly dispersible) in the aqueous-medium of the separation medium (as defined below). Capillary gel electrophoresis medium that are aqueous solutions or aqueous dispersions are preferred over non-aqueous separation medium, because they provide the necessary environment for electrophoresis (*e.g.* ions) and provide good solubility for most biomolecules of interest. Aqueous solutions also provide for ease of handling, low toxicity and cost-savings as compared to organic solvents.

[0050] Methods for preparing the polymers of the invention, as variously characterized in the above discussion, are set forth in detail below. Preferred embodiments also include polymers prepared by such methods.

[0051] Although the polymers of the present invention are suitable for use in various applications by themselves, it is also contemplated that they can be advantageously applied in various applications as blends with each other and/or with other, unrelated polymers. Hence, a further preferred embodiment of the invention is directed to blends of two or more different polymers (defined below in connection with separation media comprising different polymers), with at least one of the polymers being a polymer of the invention, as variously characterized above. Such blends can be particularly advantageous for use in separation media formulations, and especially in media for capillary gel electrophoresis. Specifically, a composition comprising such blends of polymers can be “fine tuned” with respect to particular physical and/or chemical properties of interest for a particular application.

[0052] The polymers of the invention can be used to form a polymer formulation that is used in a separation medium for fractional separation of samples having more than one component, but are preferably used as an additive to another polymer or combination

of polymers that form a separation media. When applied in connection with capillary gel electrophoresis, for example, such separation media facilitates the separation and/or analysis of a variety of biomolecules including proteins, polysaccharides and polynucleotides (*i.e.*, nucleic acid oligomers and polymers), among others. Separation media formed from the polymers described above are particularly useful for fractionating nucleic acid polymers such as deoxyribonucleic acid (DNA) polymers. While much of the discussion and examples presented herein for the separation media are directed to capillary gel electrophoresis, use in such applications is to be considered as exemplary and non-limiting, except as required in the claims. The separation medium comprises at least one polymer or blend of polymers in an aqueous medium.

[0053] The particular polymer (or blend of polymers) to be employed in the separation media can be selected to achieve particular desired properties of the separation medium (*e.g.*, viscosity) as well as particularly desired capabilities (performance characteristics) of the separation media. For example, the polymer(s) of the separation media can be selected (tailored) to achieve a particular resolution, throughput or peak capacity for a particular sample or sample fraction (*e.g.*, a polynucleotide having a specific number of base pairs). One preferred polymer in the separation media is linear polyacrylamide (LPA) having a molecular weight between about 750,000 and 2,000,000.

[0054] The aqueous medium comprises water, and preferably at least about 50% water by weight relative to total weight of the aqueous medium. The weight-percentage of water in the aqueous medium can generally range from about 50% water to about 100% water. Water can be combined with water-miscible liquids, such as methanol or other alcohols. The aqueous medium can also be a buffer or an electrolyte solution. Aqueous buffer solutions having a pH range suitable for the sample molecules of interest. For DNA separation, for example, pH can range from about 6 to about 9. For protein separation, the pH can vary over a larger range, depending on the particular protein of interest. Some proteins and other biopolymers can require relatively extreme pH conditions for separation.

[0055] The separation medium can be prepared by combining one or more polymers with the aqueous medium, and mixing to form an aqueous solution or an uniformly dispersed aqueous dispersion. As noted, the molecular weight of the polymer, the architecture of the polymer, the amount of polymer (*i.e.*, polymer loading) and the

nature of the aqueous medium can be controlled to provide the desired properties (*e.g.*, viscosity) and/ to provide the optimum analysis for a particular sample. Separation media formulated with star polymers, or with blends comprising star polymers, are particularly useful for fractionating polynucleotides by capillary electrophoretic methods.

5 **[0056]** The polymer loading in the separation media is not narrowly critical, but can be important in some cases – with respect to performance criteria (*e.g.*, sample resolution, throughput and/or peak capacity) and/or properties of interest (*e.g.*, viscosity, solubility, dispersibility and/or flowability). For applications directed to capillary gel electrophoresis, the polymer loading should be sufficient to provide adequate resolution
10 for the sample fraction of interest, without adversely affecting flowability and/or other desirable properties of the separation media.

[0057] Polymer loading is generally the amount of polymer formulation in the separation media. Polymer loading, in combination with other properties, especially molecular weight and viscosity of the separation medium, can be an effective control
15 parameter for performance features (*e.g.*, flowability, sample throughput, *etc.*). For capillary gel electrophoresis, for example, the high-end loading limit may be functionally constrained by viscosity, solubility and/or more generally, flowability. In general, for such applications, the separation media comprises polymer formulation (in any of the embodiments discussed above) at a loading of at least about 1 %, more preferably at least
20 about 3 %, and most preferably at least about 5 %, in each case the percentage being by total weight of polymeric components relative to total weight of the separation media. Surprising, while implementing this invention it was unexpectedly found that the weight fraction of wall-coating function could be much lower than is commonly found in prior art compositions, with the amount of wall-coating functioning polymer comprising about
25 0.05% by weight to about 1% by weight, more specifically between about 0.1% and 0.75% by weight and even more specifically between about 0.1% and 0.5% by weight of the separation media. Overall, rate of use of wall coating segment (in wt-% referred to the full polymer composition) is between about 0.2 % up to 20%, preferably 1.5% up to 10%.

30 **[0058]** The wall coating polymer is a polymer introduced in a smaller percentage in the formulation (typically 0.2 %w/w) known to suppress the electroosmotic flow in addition to the polymer introduced in the formulation which has to capability to separate

the DNA fragments by capillary electrophoresis but that do not provide any coating of the capillary.

[0059] The viscosity of the separation medium is preferably suitable for the application of interest, and in preferred embodiments, suitable for capillary gel electrophoresis. In particular, the viscosity of the separation medium should be controlled or adjusted such that the separation media is a flowable medium at the analysis temperature – typically about 50 °C, and in some cases preferably up to about 60 °C or higher for capillary gel electrophoresis. As used herein, the term “flowable medium” means generally, a medium that can flow under a motive force (*e.g.*, pressure head developed by a pump) through a capillary with an internal diameter or width of the system in which it will be used – typically of not more than about 100 µm. Other parameters, such as the temperature at which the separation / analysis of sample molecules is effected, polymer loading, and degree of solubility could each, independently and cumulatively, also have an effect on viscosity and/or flowability at a given polymer loading. The viscosity and, independently and cumulatively, the flowability of the separation medium are preferably suitable for capillary gel electrophoresis in a system comprising a capillary having an interior diameter or width of about 100 µm or less, preferably of about 75 µm or less, more preferably of about 50 µm or less, still more preferably of about 25 µm or less, even more preferably of about 10 µm or less, and most preferably of about 5 µm or less. As such, the viscosity of the separation medium is suitable for microelectrophoretic applications. Characterized with respect to other aspects of particular importance with respect to automation, for example, the viscosity is suitable for filling, flushing and refilling the separation medium from such capillaries (*e.g.*, for stationary separation-medium systems) and/or for flowing within the capillary (*e.g.*, for flow or counter-flow systems) at the molecular weights and polymer loadings of interest.

[0060] In one embodiment, the separation media comprises one or more polymers of the present invention (such as defined by one or more of Formulas 1, 2 and 3) that is at least partially soluble in water or aqueous medium, or dispersible in water or aqueous medium (*e.g.*, running buffer). Such polymer(s) are combined with (*e.g.*, solubilized or dispersed in) an aqueous medium to form a solution having a low polymer loading – preferably less than about 10 %, and more specifically less than 5 % by total

weight of polymer relative to total weight of the separation medium – and with a viscosity suitable for capillary gel electrophoresis.

[0061] In another embodiment, the separation media comprises a blend of polymers, including at least one sieving polymer (such as LPA) and one or more polymers of the present invention (such as defined by one or more of Formulas 1, 2 and 3) with the blend of polymers being at least partially soluble in water or aqueous medium, or dispersible in water or aqueous medium. Other known sieving polymers can be used, such as those described in U.S. Patents 5,264,101, 5,948,227, 5,567,292 and 5,885,432, each of which is incorporated herein by reference. Such polymer(s) are combined with (*e.g.*, solubilized or dispersed in) an aqueous medium to form a solution having a low polymer loading – preferably less than about 10 %, and more specifically less than 5 % by total weight of polymer relative to total weight of the separation medium – and with a viscosity suitable for capillary gel electrophoresis. As used herein, the term “different polymers” means that the polymers differ with respect to composition, chain length (*e.g.* especially for linear polymers), architecture (*e.g.*, especially for non-linear polymers), crystallinity, and/or hydrodynamic volume. The sieving polymer can be chosen from those known to those of skill in the art, but are generally polymers made from the same monomers as segment A of the polymers of this invention. Thus a formulation of this embodiment may be:

1-10% w/w polymer formulation

90-99% w/w aqueous solution,

with the above polymer formulation comprising one of the embodiments of this invention. For example, the polymer formulation can comprise one or more sieving polymers and about 0.01-0.5 wt % of the copolymer of this invention (*e.g.*, Formulas 1, 2, 3 or combinations thereof).

[0062] In another embodiment with a blend of polymers, the separation media for capillary gel electrophoresis comprises three or more different polymers – at least one sieving polymer, at least one wall coating polymer and a third polymer, with the third polymer being one or more polymers of the present invention (such as defined by one or more of Formulas 1, 2 and 3). Each of such polymers are combined with (*e.g.*, solubilized or dispersed in) an aqueous medium to form a solution with a viscosity suitable for capillary gel electrophoresis. The sieving polymer(s) can be chosen from

those known to those of skill in the art, but are generally polymers made from the same monomers as segment A of the polymers of this invention, as discussed above. The wall coating polymer(s) can be chosen from those known to those of skill in the art, but are generally polymers made from the same monomers as segment B of the polymers of this invention, as discussed above.

[0063] Also, the separation media may include other components that are commonly employed for different applications. Other components may include buffering agents or solutions, denaturing agents and the like. See, e.g., U.S. Patents 5,885,432, incorporated herein by reference.

[0064] Capillary gel electrophoresis systems are commercially available from a variety of manufacturers including, e.g., Perkin Elmer Applied Biosystems, Beckman, and Molecular Dynamics. Capillary gel electrophoresis instruments generally include a capillary capable of being connected at opposite ends to opposing polarity terminals of a voltage source, a detector, a sampling mechanism, and a separation medium disposed within the capillary. The geometry of the capillary is not critical. Typically, the capillary can be a tube (e.g., a silica tube) and/or a channel (e.g., formed in the surface of a substrate).

[0065] The capillaries of the present invention can have an inner diameter or width ranging from about 5 μm to about 200 μm , preferably ranging from about 5 μm to about 100 μm , and more preferably ranging from about 10 μm to about 75 μm . In particular, the s preferably have a diameter or width of not more than about 100 μm , preferably not more than about 75 μm , not more than about 50 μm , not more than about 25 μm , not more than about 10 μm , and not more than about 5 μm . Such capillaries can be closed or open capillaries, and can have a cylindrical-shaped interior geometry or a non-cylindrical interior geometry (e.g., oval, square, triangular, parallelogram). Such geometries may be dictated (or even preferred), for example, due to the fabrication techniques (e.g., etch angles associated with microfabrication). Hence, more generally, the capillaries can have a hydraulic radius (*i.e.*, cross-sectional area divided by circumference (or partial circumference for open channels) ranging from about 0.25 μm to about 50 μm , preferably ranging from about 0.25 μm to about 25 μm , and more preferably ranging from about 2 μm to about 20 μm . In particular, the capillaries preferably have a hydraulic radius of not more than about 25 μm , preferably not more

than about 20 μm , not more than about 15 μm , not more than about 10 μm , not more than about 2 μm , and not more than about 0.25 μm . The length of the capillary can range from about 1 cm to about 100 cm, and preferably from about 10 cm to about 100 cm. The capillaries are often made of fused silica. Flow of the separation medium can be
5 restricted by, e.g., the use of a frit or constricted plug, which prevents the flow of the separation medium out of the tube.

[0066] According to one aspect of the present invention, even relatively small-diameter or width capillaries – e.g., having an interior diameter or width of not more than about 75 μm (and preferably less, with incrementally smaller diameter or widths /
10 hydraulic radius as described above) can be filled, flushed and refilled. Hence, the capillary gel electrophoresis system can comprise a fill port for providing separation medium to one or more capillaries, a flushing port for removing spent medium therefrom, a motive force source (e.g., pump) for effecting movement of the separation media into and/or out of the capillary, and optionally, and preferably, a process control
15 system for controlling such operations. Such a process control system can include a computer, software providing a control logic, and one or more control elements (e.g., valves, pump speed controller, etc).

[0067] In operation, the separation medium is pumped into the tube so as to fill the tube with the separation medium. Volumetric flowrates for filling the tube are
20 controlled by the pump speed to range, generally, from about 50 $\mu\text{l}/\text{min}$ to about 100 $\mu\text{l}/\text{min}$. The separation medium should fill the tube substantially uniformly and homogeneously, such that voids or discontinuities that would interfere with the sample analysis are minimized, and preferably substantially nonexistent. Typically, the medium is stationary (not flowing) during the analysis. In some applications, however, flow – in
25 the same direction as sample-molecule migration or counter-current thereto – can be maintained through the capillary throughout the analysis. The sample(s) to be analyzed can be placed into the separation media prior to loading such media into the capillary, or alternatively, such sample(s) can be applied to an exposed surface of the separation media or applied to a subsurface volume of the separation media after the separation
30 medium has been established into the capillary, for example, by electro-kinetic injection. An electric field is applied across the capillary. The sample, now subjected to the electric field, migrates through the separation medium to the detector, with different

sample components migrating at different relative speeds through the separation medium. The detector provides an output signal, typically versus a time domain. The output signal is typically generated, for DNA sample fractions, by detecting tags incorporated into the particular nucleotides (A, C, T and/or G). The output signal can be correlated to the signal obtained from samples comprising standards of known fractions. For example, for analysis of DNA samples, DNA ladders with fractions having a known number and sequence of base pairs can be used for the correlation.

[0068] The capillaries of the aforementioned capillary gel electrophoresis systems preferably comprise one of the separation medium described above, alone, or in combination with other separation media.

[0069] The polymers of this invention, as variously characterized above, can be prepared by several different approaches, depending on the desired architecture and desired properties of the polymer. In general, those of skill in the art will appreciate that the polymerization processes to prepare block and graft copolymers are preferably living-type polymerization. Graft copolymers can also be prepared by the copolymerization of preformed macromonomers. Those of skill in the art can choose between the known different living type polymerization processes to find a process that best fits a particular application, with such techniques typically including nitroxide mediated, radical addition fragmentation transfer (RAFT or MADIX), iniferter and atom transfer radical polymerization (ATRP).

[0070] Polymers are prepared according to a controlled, living free-radical polymerization method, in general, by reacting a free-radical initiator with the desired monomer(s) in the presence of a free-radical control agent or other controlling technique. Solvents (*e.g.*, water) and polymerization-enhancing additives, such as accelerators or control-agent destabilizing reagents, can also be present during the reaction. A polymerization reaction mixture of this invention uses those components that are needed for the particular mechanism being practiced.

[0071] A reaction mixture comprising the free-radical initiator, monomer and free-radical control agent can be formed in a number of different ways. The control agent can be provided to the reaction mixture as a stable free-radical, or alternatively, can be formed *in situ* under polymerization reaction conditions from a stable free-radical control agent precursor. Likewise, the free-radical initiator can be provided as such to

the reaction mixture, or can be formed *in situ* under initiation conditions or under polymerization reaction conditions from a free-radical initiator precursor.

[0072] The initiators employed in the present invention can be a commercially available free-radical initiator. In general, however, initiators having a short half-life at the polymerization temperature are preferred. Such initiators are preferred because the speed of the initiation process can affect the polydispersity index of the resulting polymer. That is, the kinetics of controlled, living polymerization are such that less polydisperse polymer samples are prepared if initiation of all chains occurs at substantially the same time. The initiators are preferably water-soluble initiators and/or monomer-soluble initiators, but can also include non-aqueous solvent-soluble initiators. More specifically, suitable free radical initiators include any thermal, redox or photo initiators, including, for example, alkyl peroxides, substituted alkyl peroxides, aryl peroxides, substituted aryl peroxides, acyl peroxides, alkyl hydroperoxides, substituted alkyl hydroperoxides, aryl hydroperoxides, substituted aryl hydroperoxides, heteroalkyl peroxides, substituted heteroalkyl peroxides, heteroalkyl hydroperoxides, substituted heteroalkyl hydroperoxides, heteroaryl peroxides, substituted heteroaryl peroxides, heteroaryl hydroperoxides, substituted heteroaryl hydroperoxides, alkyl peresters, substituted alkyl peresters, aryl peresters, substituted aryl peresters, azo compounds and halide compounds. Specific initiators include cumene hydroperoxide (CHP), *t*-butyl hydroperoxide (TBHP), *t*-butyl perbenzoate (TBPB), sodium carbonate peroxide, benzoyl peroxide (BPO), lauroyl peroxide (LPO), methylethylketone peroxide 45%, potassium persulfate, ammonium persulfate, 2,2-azobis(2,4-dimethyl-valeronitrile) (VAZO®-65), 1,1-azobis(cyclo-hexanecarbonitrile) (VAZO®-40), 2,2-azobis(N,N'-dimethyleneisobutyramidine) dihydrochloride (VAZO®-044), 2,2-azobis(2-amidino-propane) dihydrochloride (VAZO®-50) and 2,2-azobis(2-amido-propane) dihydrochloride. Redox pairs such as persulfate/sulfite and Fe(2+)/peroxide are also useful. Initiation may also be by heat or UV light, as is known in the art, depending on the embodiment being practiced (e.g., UV light may be used for the modified iniferter or RAFT or MADIX techniques discussed herein). Those of skill in the art can select a proper initiator within the scope of this invention.

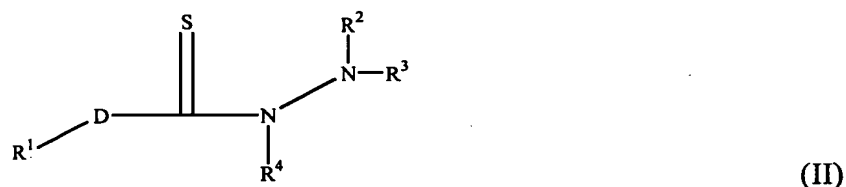
[0073] The monomers used in the polymerization process of the invention are those discussed above in connection with the polymers.

[0074] Suitable accelerators that may be included in the polymerization system include alkylating and acylating agents, Lewis Acids, ketones, aldehydes, anhydrides, acid esters, imides, oxidants and reducing agents. Specific accelerators include acetic acid, acetic anhydride, camphor sulfonic acid, acetole (1-hydroxyacetone) and the like.

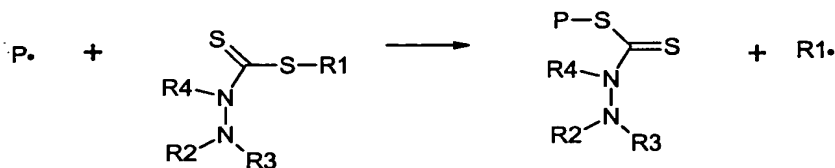
- 5 Other accelerators useful herein are recited in Hawker et al., "Development of a New Class of Rate-Accelerating Additives for Nitroxide-Mediated 'Living' Free Radical Polymerization," *Tetrahedron*, Vol. 53, No. 45, pp. 15225-15236 (1997), which is incorporated herein by reference.

- 10 [0075] Various solvents can be employed for polymerization in solution phase. Selection of solvent is dependent on the monomer, among other factors. In preferred embodiments, the polymerization is effected in aqueous solution – with the solvent being water or an aqueous medium (as defined above in connection with the separation medium). For polymerization in bulk, the monomer (or slightly diluted monomer) is the "solvent".

- 15 [0076] The control agents useful for this invention may be those known to those of skill in the art. In some embodiments that control agents may be characterized by the general formula:



- 20 wherein D is S, Te or Se. Preferably, D is sulfur. R^1 is generally any group that can be easily expelled under its free radical form ($\text{R}^{1\bullet}$) upon an addition-fragmentation reaction, as depicted below in Scheme 1 (showing D as S):



Scheme 1

- 25 [0077] In Scheme 1, P is a free radical, typically a macro-radical, such as polymer chain. More specifically, R^1 is selected from the group consisting of hydrocarbyl, substituted hydrocarbyl, heteroatom-containing hydrocarbyl, and substituted heteroatom-containing hydrocarbyl, and combinations thereof. Even more

specifically, R^1 is selected from the group consisting of optionally substituted alkyl, optionally substituted aryl, optionally substituted alkenyl, optionally substituted alkoxy, optionally substituted heterocyclyl, optionally substituted alkylthio, optionally substituted amino and optionally substituted polymer chains. And still more specifically,

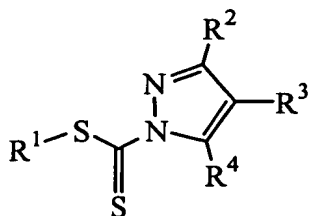
5 R^1 is selected from the group consisting of $-\text{CH}_2\text{Ph}$, $-\text{CH}(\text{CH}_3)\text{CO}_2\text{CH}_2\text{CH}_3$, $-\text{CH}(\text{CO}_2\text{CH}_2\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)_2\text{CN}$, $-\text{CH}(\text{Ph})\text{CN}$ and $-\text{C}(\text{CH}_3)_2\text{Ph}$.

[0078] Also, R^2 and R^3 are each independently selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, heteroatom-containing hydrocarbyl, and substituted heteroatom-containing hydrocarbyl, and combinations thereof. More specifically, R^2 and R^3 may be each independently selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted alkenyl, optionally substituted acyl, optionally substituted, aroyl, optionally substituted alkoxy, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted alkylsulfonyl, optionally substituted alkylsulfinyl, optionally substituted alkylphosphonyl, optionally substituted arylsulfinyl, and optionally substituted arylphosphonyl. Specific embodiments of R^2 and/or R^3 are listed in the above definitions, and in addition include perfluorelated aromatic rings, such as perfluorophenyl. Also optionally, R^2 and R^3 can together form a double bond alkenyl moiety off the nitrogen atom, and in that case R^2 and R^3 are together optionally substituted alkenyl moieties.

[0079] Finally, R^4 is selected from the group consisting of hydrogen, hydrocarbyl, substituted hydrocarbyl, heteroatom-containing hydrocarbyl, and substituted heteroatom-containing hydrocarbyl, and combinations thereof; and optionally, R^4 combines with R^2 and/or R^3 to form a ring structure, with said ring having from 3 to 50 non-hydrogen atoms. In particular, R^4 is selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted alkenyl, optionally substituted acyl, optionally substituted aroyl, amino, thio, optionally substituted aryloxy and optionally substituted alkoxy.

30 Preferred R^4 groups include methyl and phenyl.

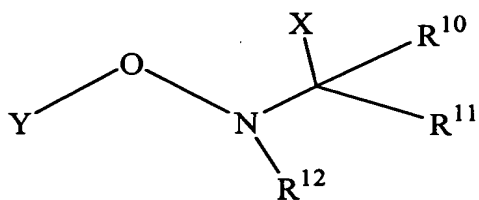
[0080] In other embodiments the control agents useful in this invention may be characterized by the formula:



(III)

wherein R^1 is defined as above and each of R^2 , R^3 and R^4 is independently selected from the group consisting of hydrogen, optionally substituted hydrocarbyl and heteroatom-containing hydrocarbyl. More specifically, R^2 may be selected from the group consisting of hydride, optionally substituted alkyl, aryl, alkenyl, alkynyl, aralkyl, alkoxy, heterocyclyl, alkylthio, amino and optionally substituted heteroatom-containing alkyl, aryl, alkenyl, alkynyl, and aralkyl. In some embodiments, R^2 , R^3 and/or R^4 may be joined in a ring structure, with the ring having from 5 to 10 atoms in the backbone of the ring (including bicyclic, tricyclic or high order ring structures). For example, R^2 may be combined with R^3 and R^3 may be combined with R^4 . Preferred control agents of Formula __ include those where R^2 , R^3 and R^4 are hydrogen.

[0081] In other embodiments, the control agents useful in making the polymers of this invention include those characterized the control agent is added to the mixture in the form of an adduct characterized by the general formula:



(IV)

Y is a residue capable of initiating free radical polymerization upon homolytic cleavage of the Y—O bond, including, for example, alkyl, substituted alkyl, alkoxy, substituted alkoxy, heteroalkyl, substituted heteroalkyl, aryl, and substituted aryl residues. X is a group capable of causing decomposition of the free radical nitroxide radical and is typically hydrogen. R^{10} , R^{11} and R^{12} are independently selected from a group consisting of alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heteroalkyl, substituted heteroalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl and silyl. Specific examples of R^{10} , R^{11} and R^{12} include methyl, ethyl, propyl, butyl, cyclopentyl, cyclohexyl, cyclooctyl, phenyl, benzyl, trimethylsilyl, those specific moieties listed in the above definitions and the like. In other embodiments using the nitroxide control agents of

this embodiment, the nitroxide can be added to the polymerization mixture as a stable free radical separate from the initiator, as is known in the art. When the adduct is employed (as shown in Formula IV), the ratio of control agent to initiator can be adjusted by the addition of free radicals from any source, including, for example, additional free radical control agent, additional free radical initiators or radicals derived from other reactions. The adducts may be prepared by methods known in the art, such as disclosed in WO 99/03894, which is incorporated herein by reference. In another such embodiment, the control agent is generated in situ from the nitron precursor, as is also discussed below and in WO 99/03894.

[0082] Control agents of these types can be prepared as is known in the art. See for example, commonly owned U.S. Patent Application No. 09/676,267, filed September 28, 2000, which is incorporated herein by reference and U.S. Patent Application No. 09/862,240, filed May 22, 2001, which is also incorporated herein by reference.

[0083] Grafted copolymers may be prepared by the macromonomer route whereby polymer chains capped at one end by a double bond reactive through free radical polymerization (*i.e.*, a macromonomer) is copolymerized with a second monomer. Methods to prepare such macromonomers are well known to those of ordinary skill in the art. One particular method that proved useful in this invention is based on the use of an addition fragmentation chain transfer agent, which allows control of the size of the macromonomers and also leaves behind a reactive double bond at one chain extremity. A typical example of such addition fragmentation chain transfer agent is the dimer of α -methylstyrene (AMSD).

[0084] A reaction mixture comprising desired monomer(s), the control agent and any initiator is formed. Typically, control agent and monomer are combined, optionally with other components, and then initiator is added as a last component to form the reaction mixture. Alternatively, the initiator and control agent can be added together as a final step (*e.g.*, as a control agent-initiator adduct). Similar protocols can be followed in bulk or in aqueous solution. The block copolymers (*e.g.*, diblocks, tri-blocks or higher order blocks, see *e.g.*, Formula 1) are typically formed by sequential addition of the block forming monomers. The graft polymers of the present invention are typically formed by forming macromers of the chain segments to be attached, and incorporating

the macromer into the backbone of the of the other segment. Thus, if the backbone segment is the sieving polymer (formula 2) and the wall coating segments are the side chains, then the side chains are polymerized first and added to the polymerization mixture during polymerization of the backbone chain. The number of chains is

5 controlled by the concentration of the macromers added to the polymerization mixture.

The macromers are made in a method known to those of skill in the art, typically using a relatively standard chain transfer method, resulting in a macromer having an unsaturated chain-end. The macromer may be isolated by methods that are known, such as by precipitation. In another embodiment, the side chains may be created by grafting from
10 the backbone by attaching a control agent to the backbone segment (e.g., segment A or segment B) and subsequently polymerizing the graft chains from the backbone.

[0085] Control of the polymerization reaction for preparing the polymers of the invention is provided by controlling various combinations of the following: selection of initiator; selection of control agent; selection of monomer; amount of control agent; the
15 ratio of initiator to control agent; the ratio of monomer to initiator; timing of addition of monomers; and polymerization reaction conditions. The monomer to initiator ratio can vary depending upon the particular architecture of interest, the desired molecular weight, the initiator efficiency and the conversion.

[0086] Polymerization reaction conditions to be controlled include temperature,
20 pressure, reaction time, and headspace atmosphere, among others. The temperature can generally range from about 0°C to about 300°C, preferably from about 0°C to about 200°C, more preferably from about 20°C to about 150°C, and most preferably from about 70°C to about 130°C. The reaction pressure can vary from atmospheric pressure to about 100 atmospheres, and preferably from about atmospheric pressure to about 10 atm.
25 The atmosphere of the reaction-vessel headspace, above the polymerization mixture, can be air, nitrogen, argon or another suitable atmosphere. The polymerization reaction time can range from about 0.1 hours to about 72 hours, preferably from about 0.5 hours to about 36 hours, and more preferably from about 1 hour to about 24 hours. The polymerization reaction conditions can be established, in general, prior to or after the
30 reaction components (free-radical initiator or precursor thereof, control-agent initiator or precursor thereof and monomer) are combined.

[0087] A number of different workup procedures can be used to modify or purify

the polymer of interest. Briefly, such approaches include: (i) precipitation, and fractionating reprecipitation of the polymers; (ii) membrane separation (*e.g.*, aqueous dialysis) of the polymers; and/or (iii) freeze-drying of the polymers. In addition, control agent molecules or fragments thereof may be cleaved from the polymer of interest in
5 ways known to those of skill in the art.

EXAMPLES

[0088] **General:** Chemicals were generally purchased from commercial sources and used as received, except either (1) as indicated below or (2) monomers, which were
10 filtered through a short column of basic aluminum oxide to remove any inhibitor and degassed by applying vacuum. All polymerization mixtures were prepared in a glove box under a nitrogen or argon atmosphere and sealed.

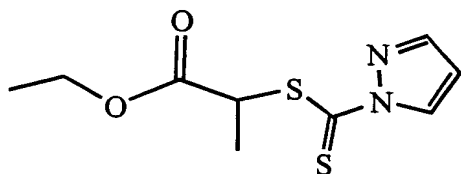
[0089] Size Exclusion Chromatography was performed using automated rapid GPC system, as described in U.S. Patents Nos. 6,175,409, 6,260,407, 6,265,226,
15 6,294,388 and 6,296,771, each of which is incorporated herein by reference. In the current setup N,N-dimethylformamide containing 0.1 % of trifluoroacetic acid was used as an eluent and polystyrene-based columns. All of the molecular weight results obtained are relative to linear polystyrene standards.

[0090] Absolute molecular weights were obtained with rapid light scattering,
20 which utilizes a combination of fast separation of macromolecules of interest from small molecules (*e.g.*, interferences) with multi-angle light scattering detection (MALS). For the fast separation, a column (50x8mm ID) was packed with microporous (30A pore size) SUPREMA-Gel, available from Polymer Standard Services (Mainz, Germany). MALS was performed on a mini-Dawn from Wyatt Technologies (Santa Barbara, CA).
25 For neutral polymers, the mobile phase was a buffer made of 0.1M sodium nitrate and 0.05wt% sodium azide in deionized water filtered carefully with 0.2 micron filters. For cationic polymers the concentration of sodium nitrate in the buffer was increased to 0.8M. Samples were dissolved in either mobile phase or in deionized water to concentration in the range 1-2g/L. Flow rate was 2mL/min generated by Waters 515
30 isocratic pump and 20uL injection volume was introduced into system by Liquid

handling robot (Cavro). Data were acquired by Astra software (Wyatt Technologies), which provides both molecular weight and radius of gyration.

Example 1: Preparation of Block Copolymer

5 [0091] The polydimethyl acrylamide first block was prepared using a "living" or controlled polymerization technique, where a polymerization mixture was formed including 2,2'-azobisisobutyronitrile as a source of radicals and ethyl 2-(pyrazole-1-carbothioyl sulfanyl) propionate as a control agent having the formula:



10 [0092] Specifically, dimethyl acrylamide was passed through an aluminum oxide activated sieves (standard grade) to remove the stabilizer. 2,2'-azobisisobutyronitrile was recrystallized in ether and ethyl 2-(pyrazole-1-carbothioyl sulfanyl) was purified by flash column chromatography.

15 [0093] Dimethyl acrylamide (9.62g, 0.0971mol) was mixed with 16.5mg of ethyl 2-(pyrazole-1-carbothioyl sulfanyl) (16.5mg, 0.0675mmol) and 0.5mg of 2,2'-azobisisobutyronitrile (0.5mg, 0.00305mmol). The reaction mixture was sealed under nitrogen in a Schlenk tube, degassed by 3 freeze-pump-thaw cycles and heated for 2h30min at 60°C to give a yellowish solid (9g, 90% yield). Samples were analyzed by rapid GPC (in DMF) and by rapid Light Scattering to get respective molecular weight of
20 190,000g/mol and 120,000g/mol. The crude product was dried under vacuum for 16-24h to remove any un-reacted monomer.

25 [0094] The solid obtained (6.479g) was dissolved into water (12.96g), 12.958g of acrylamide and 400ul of an aqueous solution of ammonium persulfate (1mg/mL) was added. The reaction mixture was sealed under nitrogen, purged by 3 vacuum/nitrogen atmosphere cycles and heated at 60°C for 1hour. The solidified reaction mixture was then dissolved in water (150mL) and precipitated into acetone (1L). The precipitate was then collected by vacuum filtration and dried to give the desired block copolymer as a white solid (15.55g, 70% yield). Molecular weight was checked by rapid Light
30 Scattering to obtain a molecular weight of 500,000g/mol.

Example 2:

[0095] In this example, separation media are prepared using a variety of polymers prepared as in Example 1. These separation media are tested for their separation performance and then aged and again tested for their separation performance.

- 5 The polymers prepared as in Example 1 are used in conjunction with a sieving polymer, which is LPA. For comparison purposes, a PDMA polymer was also used as a wall-coating polymer combined with LPA in a formulation that was prepared and aged. Each formulation for separation media for DNA separation by capillary electrophoresis was formulated by combining the components listed in Table 1, below:

10

Table 1:

Media Component	Source	W/w percentage	Weight (g)
Sieving polymer (LPA)	Applied Biosystem	2	0.1
10xGA Buffer	ABI 3700 Running Buffer with EDTA	10	0.5
Urea		38	1.9
Mill-Q water		50	2.432
Wall-coating polymer	PDMA or Polymer of this invention (e.g., Example 1)	0.2	0.01

[0096] For this example, LPA (linear polyacrylamide) was provided by Applied Biosystems and was tested prior to use (and was found to have a molecular weight of 1,500,000 g/mol as measured by Symyx Rapid Light scattering method).

[0097] There were seven formulations prepared, with 5 of the formulations using copolymers of this invention and 2 formulations using PDMA homopolymer. The properties of the "wall coating" polymers is shown below in Table 2.

[0098] The formulations were tested for their separation performance. The resulting formulations were made the day before the first test for separation performance. A vial containing the formulation was left on an asymmetric roller overnight to ensure both a proper dissolution and homogeneity of the mixture.

[0099] Separation performance was tested by running DNA separations on an Applied Biosystems, Inc. (ABI) prism 310 Genetic Analyzer capillary electrophoresis machine with polymer gels containing the separation media prepared as described above. The running buffer for the test was 10mL of 10xbuffer (ABI 3700 Running Buffer with EDTA) diluted with 90mL deionized water. A 47cm x 50µm ABI capillary with the effective length of 36 cm to the detection window was used for each separation media sample. The module used for this experiment has the following conditions: 400 seconds capillary filling with 1 ml syringe, 5 min capillary conditioning at 9.4KV, 0.5 KV injection voltage, 10 seconds injection time, 60 seconds post injection run followed by 9.4 KV running voltage and 60 minutes running time. Data was collected with frequency of 4hz. DNA separation was performed on the 18 fragments DNA having the following lengths 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, 500, 550, 600, 650, and 700 base pairs. Four injections were performed for each separation



media sample at 50°C. 2uL of the DNA sample solution provided were diluted with 18uL formamide solution, heated at 95°C for 2min. Both the buffer and the DNA were made freshly everyday.

[0100] Data were analyzed using software from National Instruments™ Labview 5.1 version 5.1.1 to estimate the crossover point. Separation performance for the un-aged separation media is shown below in Table 2.

[0101] The separation media was then aged by placing the sealed vials on a shelf for one, two, four or five weeks at room temperature. After the designated period of storage, the formulations were used as a separation media to get crossover point and migration time values, in the same manner just discussed. Table 2, below, also lists the results and illustrates the difference of the crossover point and migration time values before and after four weeks of storage at room temperature.

[0102] PDMA (A' and B', below) were prepared by reacting 1.6mL of dimethyl acrylamide in a mixture of water /isopropanol (respectively 12.036mL /2.124mL and 11.328mL/2.832mL) in the presence of 100uL of a 10mg/mL aqueous solution of both ammonium persulfate and N,N,N',N'-Tetramethylethylenediamine. Both polymers were purified by dialysis: a lyophilization allowed the polymers as dry white powders. Molecular weights were obtained by the rapid Light Scattering method described above.

Table 2:

Wall coating agent	Sample	Mw PDMA (First block)	Total molecular weight	Un-aged Performance		4 weeks Aged	
				COP	MT	COP	MT
PDMA	A'	250000	250000	660	43.7	570	37.6
	B'	177500	175000	625	39.8	570	36.7
Block Co-Polymer	A	68600	290000	646	42.6	615	40.7
	B	68600	571700	669	43.4	629	40.4
	C	68600	718800	694	46	643	43
	D	155000	1200000	673	47.5	672	44.7
	E	120000	500000	659	45.3	615	42

[0103] The separation performance as measured by cross over point (COP) and migration time (MT) is in some cases substantially the same (especially where the sieving block is of high molecular weight).

Example 3:

[0104] In this example, several formulations were prepared to show the effect of increasing the concentration of the polymers of this invention in a polymer formulation used in a separation media. In this example, di-block copolymers of PDMA and LPA are prepared (as exemplified in Example 1) and used in varying amounts in a separation media formulation, with the separation media formulation being shown in Table 1, above. Diblock copolymers made as in Example 1 were formulated at 0.1% (5mg of polymer), 0.2% (10 mg of polymer) and 0.6% (30mg of polymer). The formulations were tested for separation performance in the manner described above in Example 2. The values of the crossover point values were compared to PDMA previously described as wall coating agents 9340131 and 9340141:

Table 3 shows the polymers used and the results:

Reference	Formulation		0.1%		0.2%		0.6%	
	Mw PDMA	Mw diblock	COP	MT	COP	MT	COP	MT
9340131	250000	-	634	42.3	660	43.7	737	50.9
9340141	177500	-	385	30.3	625	39.8	691	47.5
8722021	68000	645000	677	43.1	674	44.6		
8722032	32000	485000	689	44	693	43	700	54.6
8723012	68000	570000	680	43.8	669	43.4		
8723014	68000	720000	688	45.5	721	46.2	640	54.8
8729351	100000	1200000			673	47.5		

Example 4:

[0105] Stability of the formulations involving diblock copolymers was checked by centrifugation experiments. Diblock copolymer 8723012 (Mw PDMA first block was 68,600 g/mol, total Mw 570,000 g/mol) was chosen as an example. Formulations were prepared as in Example 2, including a 2% solution of ABI-LPA and a 0.2% solution of wall coating polymer (PDMA or diblock copolymer).

[0106] Freshly prepared formulations were left on a asymmetric roller for at least 16h to ensure both a proper dissolution of the polymers and to get homogeneous mixtures. 1.6g of the formulations were introduced into a 1.7mL microcentrifuge tubes.

Centrifuge speed was chosen to be 10,000rpm (equivalent to 5585g) for 30 min on a micro-centrifuge device. Vials were removed from centrifugation device and put on a rack overnight.

[0107] Aliquots from the centrifugated formulations were taken at different level of the microcentrifuge tube. Different levels of sampling (z1, z2, z3,z4 and z5) from the centrifuged formulation as the origin were defined as:

Z1: 10 first mg from the top of the vial are taken.

Z2: 2mm from the original top of the vial are taken.

Z3: sampling is made at 1cm from the original top of the vial (70mg taken) .

Z4: 2cm from the original top of the vial (70mg taken).

Z5: is the 50 mg left of the formulation.

[0108] Formulations used as an example for comparison are typically:

S1: solution 2% LPA – 0.2 % PDMA (1000000g/mol)

S2: solution 2% LPA - 0.2% PDMA (400000g/mol)

S3: solution 2% LPA – 0.2% PDMA (200000g/mol)

S5: solution 2% LPA – 0.2 % diblock copolymer 8723012

[0109] Aliquots taken were dissolved in 500ul of D₂O and analyzed by ¹H NMR. Integration of peaks related to hydrogen atoms from methyl groups in PDMA and the ones from the polyacrylamide backbone allowed to get a molar percentage of dimethylacrylamide units versus acrylamide units in the mixture. The percentage indicated in Table 4, below shows the percentage of dimethylacrylamide units in comparison to the % of the acrylamide units. Results show an excess of dimethyl acrylamide units when sampling at the top of the formulation illustrating heterogeneity of the formulation once centrifuged.

Table 4:

Z (cm) Level of sampling from the surface	S1	S2	S3	S5
0.01	13			7
0.1	9.5	10.5	10	6
1	6.5	9.5	10	6.5

2	4.5	9.5	8.5	6
2.8	4	9.5	8	6.5

Example 5:

[0110] This example describes the preparation of graft copolymers LPA-graft-PDMA as a separation media for DNA separation by capillary electrophoresis. Synthesis of a PDMA macromonomer is carried out in a glove box. In a pressure glass vessel containing a magnetic stirring bar was added 1.5 ml of dimethylacrylamide, 0.24 ml of 2,4-diphenyl-4-methyl-1-pentene, 28 μ l of t-butylperbenzoate, and 1.5 ml of toluene. The vessel was tightly closed and was heated for 3 hr at 130 °C. The mixture was then diluted with 5 ml of dichloromethane and precipitated in hexane. The resulting solid was re-dissolved in dichloromethane and precipitated again in hexane. (Mw = 11800g/mol).

[0111] In a reaction vessel were mixed 882 mg of acrylamide, 18 mg of the macromonomer obtained, 0.3 ml of a ammonium persulfate solution (stock solution: 100 mg in 10 ml of water) 0.3 ml of a TEMED solution (stock solution: 100 mg in 10 ml of water) and 9 ml of water. The mixture was stirred at RT for 16 hr. The mixture was then diluted ten-fold times with water, purified by dialysis during 2 days. The resulting graft copolymer was obtained after lyophilization. The molecular weight was 2.1 million as measured by rapid light scattering.

[0112] In light of the detailed description of the invention and the examples presented above, it can be appreciated that the several objects of the invention are achieved. The explanations and illustrations presented herein are intended to acquaint others skilled in the art with the invention, its principles, and its practical application. Those skilled in the art may adapt and apply the invention in its numerous forms, as may be best suited to the requirements of a particular use. Accordingly, the specific embodiments of the present invention as set forth are not intended as being exhaustive or limiting of the invention.

[0113] All references, patents, articles, etc. cited herein are incorporated herein by reference for all purposes.

What is claimed is:

1 1. A separation media, comprising:

2 a polymer characterized by one of the following formulas:

3 $((A)-(B))_n-A_m$ Formula 1;

4 $(A)-(B)_{n'}$ Formula 2;

5 $(B)-(A)_{n'}$ Formula 3;

6 wherein n is at least 1; and m is 0 or 1; n' is at least 1;

7 A represents a polymeric segment prepared from one or more monomers

8 selected from the group consisting of acrylamide, methacrylamide, N-

9 alkylacrylamide, N-alkylmethacrylamide, N,N-dialkylacrylamide, N,N-

10 dialkylmethacrylamide, N-methylolmethacrylamide, N-ethylolmethacrylamide, N-

11 methylolacrylamide, N-ethylolacrylamide, hydroxyethylcellulose,

12 hydropropylcellulose, and polyethylene oxide;

13 B represents a polymeric segment prepared from one or more monomers

14 selected from the group consisting N,N-dialkylacrylamide, ethyleneoxide,

15 propyleneoxide, N-vinyl pyrrolidone, vinyl alcohol, allyl glycidyl ether, and

16 combinations thereof; and

17 A is prepared from at least one monomer that is different from at least one

18 monomer in B.

1 2. The separation media of claim 1, further comprising least one other polymer

2 in sufficient quantity to cause sieving of a biological molecule.

1 3. The separation media of claim 1 further comprising at least one other polymer

2 in sufficient quantity to cause wall coating of an uncoated capillary during a capillary

3 electrophoresis experiment.

1 4. The separation media of claim 1 further comprising a sieving polymer.

1 5. The separation media of claim 1 further comprising a wall coating polymer.

1 6. The separation media of claim 1 further comprising a sieving polymer and a

2 wall coating polymer.

1 7. The separation media of either of claims 4 or 6, wherein the sieving polymer



2 is made from the same monomer or monomers as polymeric segment A.

1 8. The separation media of either of claims 5 or 6, wherein the wall coating
2 polymer is made from the same monomer or monomers as polymeric segment B.

1 9. The separation medium of claim 7, wherein the sieving polymer is a linear
2 polyacrylamide.

1 10. The separation media of claim 1, wherein said polymer is a di-block
2 copolymer of Formula 1.

1 11. The separation media of claim 10, wherein segment A is prepared one or
2 more monomers including at least N,N-dimethylacrylamide and segment B is
3 prepared one or more monomers including at least acrylamide.

1 12. A capillary filled with an electrophoresis separation medium comprising:
2 about 0.01 % to about 5 % w/w of a polymer characterized by one of the
3 following formulas:

4 $((A)-(B))_n-A_m$ Formula 1;

5 $(A)-(B)_{n'}$ Formula 2;

6 $(B)-(A)_{n'}$ Formula 3;

7 wherein n is at least 1; and m is 0 or 1; n' is at least 1;

8 A represents a polymeric segment prepared from one or more monomers
9 selected from the group consisting of acrylamide, methacrylamide, N-
10 alkylacrylamide, N-alkylmethacrylamide, N,N-dialkylacrylamide, N,N-
11 dialkylmethacrylamide, N-methylolmethacrylamide, N-ethylolmethacrylamide, N-
12 methylolacrylamide, N-ethylolacrylamide, hydroxyethylcellulose,
13 hydropropylcellulose, and polyethylene oxide;

14 B represents a polymeric segment prepared from one or more monomers
15 selected from the group consisting N,N-dialkylacrylamide, ethyleneoxide,
16 propyleneoxide, N-vinyl pyrrolidone, vinyl alcohol, allyl glycidyl ether, and
17 combinations thereof; and

18 A is prepared from at least one monomer that is different from at least one
19 monomer in B.



- 1 13. A method of separating a mixture of biological molecules in a capillary
- 2 electrophoretic experiment with a separation media as defined in claim 1.

ABSTRACT

Graft and block copolymers are used in separation media for capillary electrophoresis separation of biological or other molecules, with the copolymers comprising one segment being made from monomers that form an effective dynamic wall coating agent and another segment being made from monomers that form an effective sieving agent. The copolymers can used alone or with other polymers that are either sieving polymers, wall coating polymers or both.